PERMEABILITY OF CERTAIN PLANT MEMBRANES TO WATER

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 230

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(WITH TWO FIGURES)

Introduction

In the exchange of material between the plant and its environment, 3 groups of substances may be considered important, namely, water, gases, and salts. These enter the plant, pass from one portion to another, and some of this material finally passes out into the environment again. In this process a great many membranes must be penetrated. The permeability of these membranes, therefore, is a factor in this material exchange, determining in a measure what substances may enter or leave the plant and at what rate this entrance or exit can take place. For this reason measurements of the permeability of plant membranes become desirable. While much work has been done toward this end from a qualitative standpoint, and while many indirect measurements have been made, direct quantitative measurements in which the results could be referred to known areas of membranes, under a standard set of conditions, have been lacking.

This paper deals with an attempt to get quantitative data on the permeability of certain plant membranes to water; to determine what laws, if any, hold for the rate of penetration of water as related (1) to temperature, (2) to direction of flow through membranes, (3) to concentration of the bathing solutions, and (4) to species of plant under consideration.

Membranes

Non-living membranes such as seed coats and the outer scale of the onion bulb were used, as they were suitable for use with the apparatus employed. The importance of non-living membranes

must not be underestimated. That they perform great physiological functions is coming to be recognized more and more as our knowledge of them increases.

The cell wall may be thought of as a non-living membrane, and its functional importance is emphasized by the work of HANSTEEN-CRANNER (16), in which it is indicated that the antagonism of $Ca^{\cdot\cdot}$ for $Mg^{\cdot\cdot}$ in root toxicity, the action of $Ca^{\cdot\cdot}$ in increasing transpiration and decreasing absorption, and the action of $K^{\cdot}$ in decreasing transpiration and increasing absorption, is due fundamentally to the effect of these ions upon the cell wall. The importance ascribed by WÄCHTER (30) to the cuticle and cork of the outer layers of the beet in preventing the loss of sugar also may be pointed out. Other investigators (4, 10) have shown that the non-living coat plays a dominant rôle in seeds, the coat character being an important factor in determining the respiration, water intake, entrance of toxic materials, delay in germination, longevity, protection from leaching of stored materials, and from mechanical injury, etc.

A study of the permeability of such membranes is desirable in itself, and it was hoped that results so obtained would throw light upon the problem of the permeability of plant membranes in general. In this connection we quote PFEFFER (24): "the physiological process itself will first have to deal with the experimental study of lifeless material, studies which may perhaps in their turn make clear processes taking place in the organism."

A non-living semipermeable plant membrane was discovered in 1907 by BROWN (5) in the barley grain, and later BROWN and WORLEY (6) measured its permeability to water. SCHROEDER (27) reported a similar membrane in the wheat grain. GOLA (13) found such membranes in the seeds of a great many different species. SCHROEDER and GOLA did not measure the permeability of the membranes to water. SHULL (28) found that the seed coat of *Xanthium* is semipermeable to certain substances, and pointed out the distinct advantage this membrane had for experimental purposes, in that it could be removed from the seed, and its permeability characters studied directly, without other structures becoming factors in the experiment. He constructed an osmometer

in which a portion of the seed coat was used as the membrane, and made preliminary measurements on the rate of penetration of water. The problem of getting quantitative measurements of the permeability of various plant membranes was then undertaken by the writer with the results here reported.

The rate of penetration of water through membranes was measured with an osmometer of the design shown in figs. 1 and 2. *A*, *B*, and *C* are hard rubber discs, 3.5 cm. in diameter and 2 mm. in thickness. Brass discs also were used, but are not suitable for

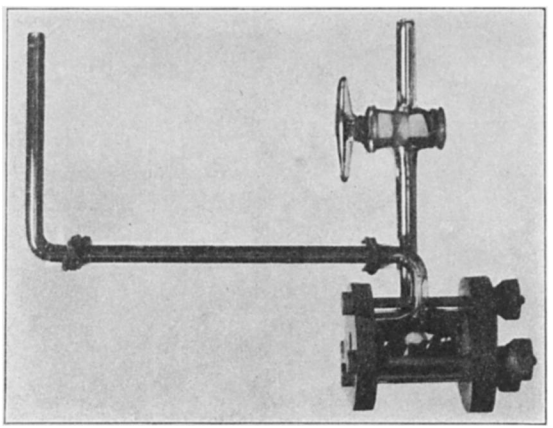

FIG. 1.—Photograph of osmometer: explanation in text

use with salt solutions. In the centers of *A* and *B* at *K* is a hole of known diameter. Between *A* and *B* and over this hole the membrane to be studied is placed; thus the area of the membrane used can be calculated. *D* is a hard glass cylinder with ground edges fitting snugly against the hard rubber discs. Soft rubber gaskets are interposed between the glass cylinder and the discs at *E*, and the apparatus made tight by screwing up the bolts at *H*. F is the tube for admitting water into the internal chamber. The latter is filled with distilled water until water appears in the horizontal capillary tube. The position of the meniscus in *G* may be set at any desired spot by means of the stopcock in *F*. *G* is a capillary tube with about 10 cm. horizontal length and with a capillary bore of approximately 1 mm. Scale divisions on *G* were

calibrated by weighing with mercury. One scale division on G = 0.000337 gm. of water at 25° C. The whole apparatus is then immersed in a vessel containing a solution of cane sugar or sodium chloride and the vessel placed in a water bath regulated to constant temperature. The osmotic force of the bathing solution pulls water through the membrane from the internal chamber, and this causes the meniscus in the capillary tube to recede. By successive

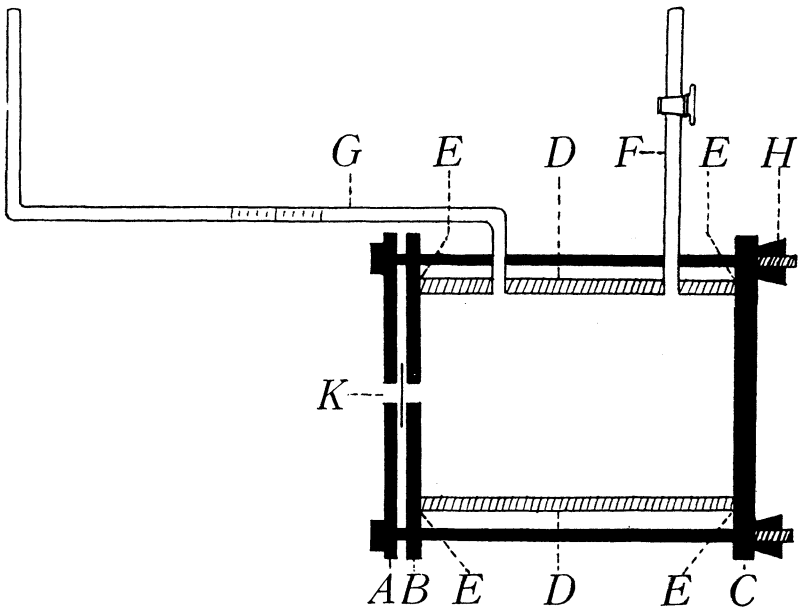

FIG. 2.—Drawing of osmometer: explanation in text

readings of the position of the meniscus in G at various intervals of time the rate of water movement through the membrane can be determined.

As water passes through the membrane, it has a tendency to dilute the bathing solution at K. This tendency is overcome by a stirrer whirling in front of the membrane at K which keeps the concentration constant there. The amount of water passing into the bathing solution is so small as compared with the large volume of the latter that the concentration of the solution exerting the osmotic pull is maintained constant.

At the end of an experiment it is possible to record the quantity of water passing through a known area of membrane, in a known interval of time, at a constant known temperature, and under the constant osmotic pull of a solution whose osmotic pressure in atmospheres is known. This gives a measure of the permeability of the membrane under the conditions of the experiment.

The sources of error and the precautions taken were as follows:

Temperature errors.—It was found that when the apparatus was immersed in the bathing solution it reached the temperature of the latter in 6–8 minutes. An interval of at least 10 minutes was allowed before readings were begun. The temperature of the bathing solution was constant to $\pm 0°.1$ C. The effect of the possible deviation of the temperature of the internal liquid upon the readings of the meniscus in the side arm was calculated.[1] The volume of the osmometer is 4421.13 cu. mm. Assuming it filled with water at 4° C., then changes in temperature of $0°.1$ show the relation between temperature errors and scale division of the osmometer as indicated in table I.

TABLE I

Temperature	Volume error (in mg.) of water	Scale division value (in mg.) of water	Error of readings in scale divisions
$5° \pm 0°.1$.....	0.01326	0.338	<1
$15° \pm 0°.1$.....	0.17665	0.3378	<1
$25° \pm 0°.1$.....	0.18525	0.337	<1
$35° \pm 0°.1$.....	0.30764	0.336	<1
$45° \pm 0°.1$.....	0.37212	0.3347	1.11

Accurate readings of the side arm could be made only to one scale division. Temperature deviation therefore introduced no error except at temperature as high as 45°, and then only a slight error resulted.

Variation in membranes.—It was early found that there was a large variation in the permeability of different membranes of the same species. One membrane could not be compared with another, but only with itself under the various conditions of the experiment.

[1] Calculated from data taken from Smithsonian tables, Smithsonian Misc. Coll. **63**: no. 6.

The permeability of the same individual membrane was measured under the different conditions studied, therefore, and the same set of observations made with a number of other membranes of the same species. In some cases it was possible to allow the same membrane to remain in the osmometer during a whole series of readings. When it was necessary to remove the membrane from the osmometer, care was taken in replacing it that the same portion of the membrane was used in the next reading.

Constancy of semipermeability.—When a membrane gave constant rates for 1 hour, readings being taken at intervals of 10 minutes, it was assumed that its permeability to salt or sugar had not changed during the experiment, or at any rate that any change in permeability that had occurred did not affect the readings taken. No serious attempt was made to determine the completeness of semipermeability. Preliminary experiments indicated that the membranes were slightly permeable to sodium chloride, but a passage of cane sugar through the membrane was not detected. Conductivity measurements are to be made to determine the permeability of these membranes to salts, and these results will be reported in a later paper.

Preparation of solutions.—Cane sugar solutions were prepared in accordance with tables given by FINDLAY (11), rock candy being used. Sodium chloride was made up on the volume molecular basis and its osmotic pressure figured from the data given by RENNER (25).

Capillary tube errors.—Although according to the law of POISEUILLE the flow of water through capillary tubes is affected by temperature, it is not believed that this was a factor in these experiments. In POISEUILLE'S experiments the liquid was subjected to a head of pressure and flowed through the capillary tube with rapidity, whereas in these experiments there was no hydrostatic pressure applied and the rate of movement through the tube was very slow. According to BARKER (3), when water is the liquid in question this law applies only to tubes with less than 0.5 mm. diameter of capillary; while the capillary used in these experiments was 1 mm. It is not believed that the results obtained are affected by the influence of temperature on the flow of water through the capillary tube of the apparatus.

Other precautions taken.—All membranes were heated in boiling water before being used for experimental purposes. The distilled water used inside the osmometer was previously boiled to drive off dissolved gases. Tests were made to determine that no leakage was occurring in the apparatus. In filling the osmometer care was taken to drive out all air bubbles from the internal portion of the apparatus. Special pains were taken to see that no air bubbles were lodged on the membrane.

Effect of temperature

Membranes of the seed of *Arachis hypogaea* were placed in the osmometer and measurements were made of the rate of penetration of water at the temperatures indicated. After being measured at the various temperatures, each membrane was checked back

TABLE II

EFFECT OF TEMPERATURE UPON PERMEABILITY OF SEED COAT (MEMBRANES OF *Arachis hypogaea*)

Number	Osmotic pressure at 25°C.	Water (in mg.) passing through 19.635 sq. mm. of membrane per hour				
		5°.2 C	15°.2 C	25°.2 C	35°.0 C	45°.0 C
1	27.65	11.01	18.59	29.09	.....	.....
2	"	22.93	38.08	58.19	.....	.....
3	"	.....	.....	56.48	78.71	104.85
4	"	.....	.....	68.46	91.18	126.78
5	41.48	16.51	28.32	42.79	.....	.....
6	"	33.02	53.12	82.15	.....	.....
7	"	42.19	69.07	102.69	128.69	161.83
8	"	37.91	59.03	88.99	106.87	142.83
9	13.82	.....	.....	28.52	40.59	52.96
10	"	.....	.....	35.37	50.82	71.68
11	"	13.45	21.25	34.23	47.22	61.09
12	"	15.41	24.79	35.94	46.40	64.20

From left to right, the figures are readings obtained from the same individual membrane at different temperatures; each line represents a different membrane.

against a previous temperature to be certain of constant behavior. In transferring from one temperature to another the membrane was not removed from the osmometer, so that the results indicated in table II show a comparison of the rates indicated by the same membrane at different temperatures. When the bathing solution was changed from one temperature to another its osmotic pressure also changed, and a correction was made for this change in osmotic

pressure of the bathing solution due to changes in temperature. The actual osmotic pressure of the solution at the temperature used was calculated on the basis of proportionality between osmotic pressure and absolute temperature. The observed rate was corrected on the basis that the rate was proportional to the pull applied, which will be shown later to be the case for solutions of sodium chloride. When cane sugar was used as the bathing solution the observed rate was not corrected, because proportionality between rate and pull applied does not exist with such solutions.

TABLE III

VALUE OF Q_{10}

5.2–15°.2 C.	15.2–25°.2 C.	25.2–35°.0 C.	35.0–45°.0 C.
1.688	1.564	1.374	1.332
1.661	1.528	1.339	1.390
1.716	1.512	1.258	1.259
1.609	1.546	1.206	1.364
1.637	1.487	1.433	1.305
1.530	1.507	1.448	1.411
1.579	1.610	1.388	1.294
1.608	1.449	1.298	1.384
Average, 1.628	1.525.	1.343	1.344

TABLE IV

EFFECT OF TEMPERATURE UPON PERMEABILITY OF SEED COAT (MEMBRANES OF *Arachis hypogaea*)

Number	Osmotic pressure of cane sugar solution at 25° C.	Water (in mg.) passing through 19.635 sq. mm. of membrane per hour			
		3°.6 C	13°.6 C	23°.6 C	33°.6 C
1.....	21.25	7.98	13.69	20.54	26.81
2.....	"	13.12	21.11	30.07	42.21
3.....	"	11.64	19.40	28.92	38.79
4.....	"	6.39	10.27	14.38	19.51
5.....	"	14.55	23.39	35.09	46.89

From table II we may estimate the coefficients for 10° rise in temperature (see table III), hereafter referred to as Q_{10}. Experiments were carried out also in which cane sugar was used as the bathing medium instead of sodium chloride. The results with cane sugar are given in table IV. The figures in table IV give

coefficients for 10° C. as recorded in table V. These data were not corrected for change in osmotic pressure due to change in temperature, because, as will be shown later, the rate is not exactly proportional to the pull applied. But that this correction would

TABLE V

VALUE OF Q_{10}

3.6–13°6 C.	13.6–23°6 C.	23.6–33°6 C.
1.716	1.541	1.305
1.610	1.424	1.403
1.667	1.492	1.342
1.607	1.400	1.357
1.607	1.500	1.336
Average, 1.641	1.463	1.348

be a small one and that it would not affect the general results, is indicated by the following results obtained when a correction is figured on the assumption that the rate is proportional to the pull. Making this correction, the first column of table IV, showing values of Q_{10}, becomes:

1.649
1.547
1.604
1.545
1.546

Average, 1.578

In addition to this, preliminary results were obtained from measurements of 6 other membranes. While the conditions of the experiment were not so accurately controlled, the average coefficients obtained were as follows:

Approximately 5 to 15° $Q_{10}=1.617$
" 15 to 25° $Q_{10}=1.470$
" 25 to 35° $Q_{10}=1.422$

Temperature coefficients.—The effect of temperature upon a process has been much used to obtain information as to its nature, that is, whether chemical or physical. Generally speaking, chemical processes follow the van't Hoff law ($Q_{10}=2$ to 3), but the effect of

temperature upon the process of diffusion is such that Q_{10} is approximately 1.3. Applying the results of these experiments to this case it is found that the coefficient obtained does not correspond with either the van't Hoff coefficient or the diffusion coefficient. Measurements of the permeability of membranes made heretofore have shown in general a temperature coefficient approximating that of the van't Hoff law, but there is no evidence in these experiments that in the passage of water through the seed coat of the peanut chemical processes are exclusively involved. Apparently also the effect of temperature is not merely upon the rate of diffusion of water. Probably we are not justified in using the numerical coefficients obtained to form any conclusion as to the nature of the process by which water passes through the peanut membrane.

Comparison with temperature coefficients obtained by others.— KRABBE (**19**) measured the effect of temperature upon the permeability of the living cells of cylinders of pith of *Helianthus annuus* and pieces of roots of *Vicia Faba*, etc. As criteria he took the rate of increase in length of pieces of plasmolysed tissue, allowed to absorb water at temperatures in vicinity of 0° and 20° C, and the length of time for plasmolysis to occur at these temperatures. He found that the velocity of water movement increased 3–5 times when the temperature was increased 20° C. (Q_{10} approximately 2.0–2.5). He believed that this high coefficient indicated that purely physical forces were not operative, but that it was due to a specific property of living protoplasm.

RYSSELBERGHE (**26**) investigated the effect of temperature upon the permeability of the living protoplasm, using pith cells of *Sambucus nigra*, lower epidermal cells of *Tradescantia*, and filaments of *Spirogyra*. He made use of 3 methods: the rate of shortening of a tissue in a plasmolysing solution at different temperatures, the rate of elongation of plasmolysed tissue in water at different temperatures, and the rate of plasmolysis of a tissue under microscopic observation. His general results are as follows:

Temperature	0	6	12	16	20	25	30
Comparative rate	1	2	4.5	6	7	7.5	8

This gives an average value for Q_{10} from 0°–30° of 2.0. RYSSELBERGHE does not agree with KRABBE that this high coefficient necessarily indicated the special activity of vital matter.

BROWN and WORLEY (6) determined the speed of intake of water by barley grains immersed in water at different temperatures. Their results gave a temperature coefficient of 1.8–1.9. Since this approached closely the van't Hoff coefficient (2–3) for the effect of temperature on the rate of chemical reaction, they considered that chemical processes were involved in the penetration of water through the semipermeable membrane of the barley grain. This chemical reaction, according to their view, took place in the water itself, that the effect of temperature was to split the larger aggregates of water into simpler ones, and that only these simpler molecules were transmitted by the differential septum. This was offered as evidence in favor of the hydrone conception of ARMSTRONG (1) as to the composition of water.

PFEFFER (24) measured the rate of water movement across the copper ferrocyanide membrane at different temperatures with the following results:

Temperature	In stream per hour
7°.1	5.9 mm.
17°.6	9.4 "
32°.5	13.3 "

The above figures give values of Q_{10} as follows:

$$7.1\text{–}17°.6,\ Q_{10}=1.558$$
$$17.6\text{–}32°.5,\ Q_{10}=1.266$$

The writer's observed values are in fair agreement with these figures. For purposes of comparison, a summary is given in table VI. It will be noted from this table that no parallel exists between

TABLE VI

Observer	Nature of membrane studied	Temperature range	Q_{10}
Krabbe	Living pith cells of *Helianthus*	0–4 to 20–26°	2.0 to 2.5
Rysselberghe	Living cells	0 to 30°	2.0
Brown and Worley	Semipermeable membrane of barley seed	3.8 to 34°.6	1.9 to 1.8
Pfeffer	Copper ferrocyanide	7.1 to 32°.5	1.558 to 1.266
The writer	Seed coat of *Arachis hypogaea*	3.6 to 45°	1.641 to 1.343

the nature of the membrane studied and the value of the coefficient observed. If this were the case, the barley and peanut membranes

should be expected to give similar results, while the similarity of results given by the copper ferrocyanide membrane and peanut should not be expected. We may note, however, a parallel between the method of observation employed and the coefficient obtained. The first 3 observers studied the permeability of the membrane indirectly, other structures such as cell contents and seed contents being present. In the last two cases the membrane was measured directly, without other structures being factors in the rates observed.

It is questionable to what extent results obtained by the indirect method may be referred to the membrane alone. There is the possibility that the temperature effect may have been, not upon the membrane merely, or upon the water exclusively, but also upon the cell contents or seed contents. The latter effect may have contributed to the total results from which the coefficients were calculated. The chemical reaction indicated by the coefficient 2–3 may have taken place in that phase of the system that was internal to the membrane studied.

In these experiments the temperature may have exerted an effect on the water, but if so the temperature coefficient does not indicate that this was related to a chemical reaction. There is no evidence of a temperature action in splitting the larger water aggregates into simpler hydrone molecules as found by BROWN and WORLEY with the semipermeable membrane of the barley grain.

Tendency of temperature coefficients to fall in value with increased temperatures.—An inspection of the temperature coefficients obtained in these experiments shows that the coefficients are higher at the lower temperatures and lower at the higher temperatures. This has been found to hold for a great many different processes. KANITZ (**18**) noted a number of physiological processes that show this tendency. SNYDER (**29**) has pointed out that some purely chemical reactions also exhibit a falling value of Q_{10}, and COHEN-STUART (**8**) has shown that according to the van't Hoff law itself values of Q_{10} are not constants and that the velocity is not an exponential function of the temperature. Table VII indicates the general tendency of Q_{10} for different processes. Falling values of Q_{10} are thus shown to occur in measurements made (*a*) with living matter, (*b*) with non-living matter, (*c*) with a physical

process, and (*d*) with a chemical process. These figures also emphasize the fact that temperature coefficients should not be averaged for a large interval of temperature, but that the range of the values of Q_{10} for each temperature interval should be shown for which experimental data are available.

TABLE VII

Rysselberghe's results with living protoplasm		Results obtained with non-living plant membranes		Vapor pressure of water at various temperatures		Remsen and Reid's results with hydrolysis of nitro-benzamide*	
Temperature	Q_{10}	Temperature	Q_{10}	Temperature	Q_{10}	Temperature	Q_{10}
0–6°	3.2	5.2–15°2	1.628	5–15°	1.943	60–70°	1.84
6–12	3.8	15.2–25.2	1.525	15–25	1.854	70–80	1.72
12–16	2.0	25.2–35.0	1.343	25–35	1.776	80–90	1.65
16–20	1.5	35.0–45.0	1.344	40–50	1.675	90–100	1.59
20–25	1.1	.....	.....	.....	.....	.....	.....
25–30	1.1	.....	.....	.....	.....	.....	.....

* From data given by Snyder (**29**, p. 169).

Relations of permeability of membranes to vapor pressure of water.—The experiments of Brown and Worley (**6**) showed that Q_{10} approximated in numerical value the vapor pressure coefficients of water at those temperatures. From table VII it will be noted that similar results were not obtained with the peanut membrane; that while the coefficient of permeability rates and vapor pressure are not equal, they both show the same tendency to fall in value at higher temperatures. It may be noted in this connection that the coefficient obtained lies between the diffusion coefficient and the vapor pressure coefficient.

Rate as related to flow through capillary tubes.—According to the law of Poiseuille, as reported by Krabbe (**19**), the quantity of water flowing through a glass tube increases from 1 to $1+0.0336793\,t+0.0002209936\,t^2$, where t is the temperature in degrees Centigrade (Krabbe **19**, p. 477). This would make the coefficient for 10 rise in temperature about 1.358. Since this law applies only to capillary tubes with a length above a certain minimum amount, and since the temperature coefficients obtained in these experiments are not constants but vary with the temperature, it is not believed

that the results obtained indicate that the passage of water through the membrane is analogous to the passage of water through capillary tubes.

Rate as related to previous heating or cooling.—When the permeability of a membrane is measured at one temperature and the membrane then transferred to another temperature, the question is raised as to whether or not there is any "after effect" of the previous temperature. To determine this point membranes were fitted into 2 osmometers and a measurement was made of the permeability of each membrane. One osmometer was then placed in a beaker of water in an ice chest at 2.5 C., and the other in an oven at 46° C. The next day the two were again placed in the original osmotic solution at the original temperature and readings again taken. The results obtained are given in table VIII. No after effect of a previous temperature, or hysteresis, was observed at the temperatures used in these experiments.

TABLE VIII

Intervals of 10 minutes	25° C.	25° C.
FIRST MEMBRANE		
First	27 spaces	29 spaces*
Second	29 "	29 "
Third	28 "	29 "
Fourth	29 "	29 "
Fifth		29 "
SECOND MEMBRANE		
First	23 spaces	23 spaces†
Second	23 "	24 "
Third	23 "	24 "
Fourth	24 "	22 "
Fifth		23 "

* After 14 hours at 2°.5 C.
† After 15 hours at 45° C.

Rate as affected by direction of flow of water through membranes.—A peanut seed coat membrane was placed in an osmometer and a measurement made of the rate at which water passed through it. The membrane was then removed from the osmometer and its position reversed, the opposite surface being turned toward the

inside of the osmometer. The latter was then placed again in the original solution and a reading made of its rate of water passage. Table IX indicates the results obtained. The peanut membrane therefore is more permeable for water in one direction than in the other, and the favorable direction is from the outside toward the inside.

TABLE IX

RATE AS AFFECTED BY DIRECTION OF FLOW OF WATER THROUGH MEMBRANE OF *Arachis hypogaea*

NUMBER	DIAMETER OF HOLE	OSMOTIC PRESSURE	WATER (IN MG.) PASSING THROUGH MEMBRANE PER HOUR				
			In	Out	In	Out	Percentage decrease from in to out
1	8 mm.	67	139.52	95.92	135.16	90.91	32
2	"	"	137.34	87.20	111.18	84.58	31
3	"	"	124.26	93.09	.....	.....	25
4	"	"	99.25	43.21	98.86	.....	56
5	"	100	396.21	207.94	402.67	205.97	49
6	"	"	236.88	158.20	224.80	146.12	34
7	"	"	164.38	121.27	202.32	127.59	32
8	5 "	25	36.89	20.82	30.04	24.70	32
9	"	"	30.55	27.70	38.02	26.98	20
10	"	48	.....	70.17	106.11	.....	33
11	"	"	.....	79.87	111.82	.....	38
12	"	"	.....	35.65	53.77	.....	33
13	"	"	.....	44.75	63.69	44.50	29
14	"	"	.....	49.06	71.88	.....	31

"In" means direction outside of seed toward inside; "out" means direction inside of seed toward outside.

Measurements with the seed coats of *Prunus Amygdalus dulcis* gave the following results:

Rate in = 48.5 mg. per hour
Rate out = 42.6 " " "
Rate in = 48.5 " " "
Rate out = 40.4 " " "

Measurements made with the onion scale and with the seed coat of *Dioon edule* did not indicate any observable difference in the rate of penetration in opposite directions. This difference in the behavior of the two types of membranes may be correlated with their differences in structure. The peanut and almond seed coats are composed of two or more distinct layers, and have surfaces of different physical and chemical nature on opposite sides; such is

not the case with onion and cycad membranes. The differences in rate in opposite directions through a membrane have long been known to workers with animal membranes. MATTEUCCI and CIMA (21) in 1845 observed it with the skin of the frog and eel. COHNHEIM (9), according to HAMBURGER, ascribed the same phenomenon to the living action of the intestinal membrane. HAMBURGER (14) showed that this behavior was not restricted to living membranes, but that non-living animal membranes gave similar results; in fact, he prepared artificial membranes from parchment with layers of collodion, chromgelatin, and chromalbumen that were more permeable in one direction than in the other. He ascribes this to the "double" nature of the membrane, and the writer's results offer evidence in favor of HAMBURGER'S interpretation. If this difference in rate is due to the presence of double membranes of different nature, or to differences in surface on opposite sides, may not the plant cell itself show a difference in permeability in opposite directions, since such a system of double membranes is represented by the cell wall and ectoplast?

Rate as related to the concentration of the external solution.— Solutions of different osmotic pressures were used as the external solution in order to determine whether or not the rate of water

TABLE X

RELATION BETWEEN OSMOTIC PRESSURE APPLIED AND RATE (SODIUM CHLORIDE SOLUTION)

Number	Water (in mg.) passing through membrane per hour in atmospheres of osmotic pressure		
	13.82	27.65	41.48
1	22.53	44.37	67.89
2	21.96	44.37	66.75
3	.....	28.09	42.79
4	.....	58.18	82.15
5	28.53	56.48	.....
6	35.37	68.93	.....
7	34.23	.....	102.69
8	35.94	88.0	88.99

movement was proportional to the pull applied. Two solutions were used, sodium chloride and cane sugar. The results with sodium chloride are shown in table X. A comparison of the

osmotic pressure and rates from the data in table X is given in table XI.

TABLE XI

Ratio of pressures 27.65:13.82	Ratio of rates	Ratio of pressures 41.48:27.45	Ratio of rates	Ratio of pressures 41.48:13.84	Ratio of rates
2.000.....	1.969	1.500.....	1.530	3.000.....	3.013
2.000.....	2.020	1.500.....	1.504	3.000.....	3.040
2.000.....	1.980	1.500.....	1.471	3.000.....	3.029
2.000.....	1.949	1.500.....	1.412	3.000.....	2.977

TABLE XII

RELATION BETWEEN OSMOTIC PRESSURE APPLIED AND RATE (CANE SUGAR SOLUTION)

Number	Water (in mg.) passing through membrane in atmospheres of osmotic pressure					
	5.15	10.30	15.62	21.25	29.22	48.00
1.....	.....	23.39	29.44	33.66	.....	.....
2.....	.....	29.44	37.65	.....	.....	.....
3.....	9.70	21.11	27.09	35.37	.....	.....
4.....	8.55	18.25	24.53	31.95	.....	.....
5.....	8.16	16.83	24.25	28.43	.....	.....
6.....	7.70	13.35	18.17	21.96	.....	.....
7.....	14.09	27.04	36.62	45.18	.....	.....
8.....	14.73	25.84	35.26	42.79	.....	.....
9.....	11.81	19.40	23.96	28.24	.....	.....
10.....	12.83	23.45	31.66	35.37	.....	.....
11.....	10.84	21.11	27.95	33.66	.....	.....
12.....	13.69	24.53	31.95	39.36	.....	.....
13.....	5.53	9.69	11.98	13.69	.....	.....
14.....	6.84	11.72	13.69	16.54	.....	.....
15.....	11.98	23.96	29.66	36.97	.....	.....
16.....	.....	23.10	28.07	34.80	.....	.....
17.....	.....	.....	.....	.....	41.07	53.05
18.....	.....	.....	.....	.....	50.49	63.32
19.....	.....	.....	.....	.....	64.75	84.72

A comparison of the ratios of pressure and the ratios of the rates indicated in table XII is given in table XIII.

When the ratios of pressure applied were 2.000, 1.515, 1.360, and 1.642, the average ratio of rates observed was 1.888, 1.297, 1.211, and 1.285 respectively. It will be seen that the rate of water penetration is nearly proportional to the pull applied when sodium chloride is used, but that when cane sugar is used as the external solution, the rate is not proportional to the pull, but the

coefficient falls off with the higher concentrations. It is believed that this lowering of the rate is due to the increasing viscosity of more concentrated sugar solutions.

TABLE XIII

RATIOS OF RATES

5.15:10.30	10.30:15.62	15.62:21.25	29.22:48.0
2.176	1.259	1.144	1.292
2.133	1.279	1.305	1.254
2.063	1.284	1.302	1.308
1.734	1.344	1.172	.....
1.919	1.441	1.209	.....
1.756	1.361	1.234	.....
1.634	1.354	1.214	.....
1.825	1.364	1.179	.....
1.947	1.235	1.117	.....
1.792	1.351	1.204	.....
1.753	1.325	1.233	.....
1.712	1.302	1.143	.....
2.000	1.235	1.208	.....
"	1.168	1.246	.....
"	1.238	1.249	.....
"	1.215	1	.....
Average, 1.888	1.297	1.211	1.285

It was found that it was not possible to increase the concentration of the solutions on opposite sides of a membrane by an equal amount on each side without changing the rate at which water passed through the membrane. This was done in the following way: distilled water was placed inside the osmometer, the apparatus surrounded by a solution of sodium chloride having a known osmotic pressure, and the rate of water movement measured. Then the osmotic pressure was increased on each side of the membrane by an equal amount. The results are given in table XIII. Thus, although the effective osmotic pressure exerting an influence upon water movement was practically the same, the rate of water movement was not the same, but much less. With the same membrane the same osmotic pull does not give the same rate, but the rate depends upon the distribution of the concentration on opposite sides of the membrane. When the concentration of the external solution was kept constant and the concentration of the internal solution was varied, the results given in table XIV

were obtained with sodium chloride solutions. From these data the writer has not been able to formulate any mathematical relation between differences in concentration on opposite sides of the membrane and the rate of water movement through it. Another

TABLE XIII

RATE OF WATER MOVEMENT AS RELATED TO DIFFERENCES IN CONCENTRATION OF SOLUTIONS ON OPPOSITE SIDES OF MEMBRANE

Membrane	Solution	Osmotic pressure of external solution	Osmotic pressure of internal solution	Effective osmotic pressure	Rate per hour
First.....	Na Cl	18.43	0	18.43	48.29
"	"	36.86	18.43	18.43	35.94
Second.....	"	13.82	0	13.82	41.07
"	"	27.65	13.82	13.83	23.96
Third.....	Sugar	10.30	0	10.30	20.54
"	"	21.25	10.30	10.95	10.84
Fourth.....	"	10.30	0	10.30	29.09
"	"	21.25	10.30	10.95	15.63

TABLE XIV

FALL IN RATE OF WATER MOVEMENT WHEN CONCENTRATION OF INTERNAL SOLUTION WAS INCREASED

OSMOTIC PRESSURE OF EXTERNAL SOLUTION	OSMOTIC PRESSURE OF INTERNAL SOLUTION	WATER (IN MG.) PASSING THROUGH MEMBRANE PER HOUR	
		First	Second
46.10.....	0	67.74	61.67
"	4.61	49.54	45.21
"	9.22	45.49	39.43
"	13.82	39.39	32.99
"	18.43	26.96	24.27
"	23.05	22.92	18.86
"	27.65	12.13	10.11

set of readings was taken in which cane sugar solutions were used. The results are given in table XV, and show that the relation between concentration and rate is complex.

From the data given in tables XIII–XV we may conclude: (1) that the rate is greatly affected by changes in the concentration of the internal solution; (2) that equal osmotic differences do not necessarily produce equal rates; and (3) that no mathematical relation has been noted between the concentration on opposite

sides and the rate of water movement through the membrane. This emphasizes the caution that must be used in plasmolytic experiments on the rate of water movement through a membrane. Plasmolysis deals with solutions of different concentrations on opposite sides of a membrane. The concentration of only one of the solutions, the plasmolysing solution, is known. In such experiments the internal concentration of the cells of plant or seed is not known and is subject to change, that is, to variations in pulling power. Results should not be referred to changes in the permeability of the membrane alone until it has been found that the internal concentration has remained constant during the experiment (it is to be understood that this statement is intended to apply to *rate of water movement* and not to the final equilibrium attained by the two solutions).

TABLE XV

RATE OF WATER MOVEMENT AS RELATED TO DIFFERENCES IN CONCENTRATION OF SOLUTIONS ON OPPOSITE SIDES OF MEMBRANE

OSMOTIC PRESSURE OF EXTERNAL SOLUTION	OSMOTIC PRESSURE OF INTERNAL SOLUTION	EFFECTIVE OSMOTIC PRESSURE	WATER (IN MG.) PASSING THROUGH MEMBRANE PER HOUR	
			First	Second
73.69.....	0	73.69	41.07	37.65
48.0.....	0	48.0	37.65	34.23
21.25.....	0	21.25	27.38	21.39
10.30.....	0	10.30	13.59	17.11
73.69.....	10.30	63.39	21.39	20.54
73.69.....	21.25	52.42	9.58	11.13
48.0.....	10.30	37.70	17.11	16.26
21.25.....	10.30	10.95	6.84	8.56

Comparison of permeability of membranes of different species

Membranes from different species showed large differences in permeability, as indicated by table XVI. Equal areas (19.635 sq. mm.) of membranes were measured; saturated sodium chloride of approximately 375 atmospheres osmotic pressure was used as the external solution. It will be seen that membranes of different species and different membranes of the same species show large differences in permeability. The causes of these differences in the

rate of penetration will be dealt with in a later paper. It may be stated here, however, that thickness of membrane is not the limiting factor. The thinnest membrane is that of *Cucurbita*, and the thickest is that of *Prunus Amygdalus*.

TABLE XVI

RELATIVE PERMEABILITY OF VARIOUS MEMBRANES

Membrane	Water (in mg.) passing through per hour	Membrane	Water (in mg.) passing through per hour
Citrus grandis	0	Allium Cepa	39.2
" "	0	" "	12.9
" "	0	" "	12.3
" "	0	" "	31.2
Cucurbita Pepo	7.3	" "	22.4
" "	4.8	Prunus Amygdalus dulcis	120.0
" "	0	" " "	144.0
" "	4.0	" " "	72.0
" maxima	11.0	" " "	86.0
" "	0	" " "	60.0
" "	9.0	" " "	72.0
" "	11.3	" " "	72.0
" "	15.3	Arachis hypogaea	328.0
Xanthium pennsylvanicum	20.0	" "	530.0
" "	16.0	" "	564.0
" "	14.7	" "	710.0
" "	22.0	" "	528.0
Juglans regia	32.0	" "	672.0
" "	22.7	" "	584.0
Allium Cepa	25.7	Dioon edule	777.5

Structures of membrane used

The layers of tissue represented in the ripened seed coat of the various species and their origin have not been accurately determined by an examination of successive stages of the development of the seed. A study of the histology of the seeds of *Cucurbita* has been made by BARBER (2), of *Prunus Amygdalus* by PÉCHOUTRE (23), of *Xanthium* by HANAUSEK (15), of the Leguminosae by PAMMEL (22), and of the seed coats of various species in many families by LONAY (20), BRANDZA (7), GUIGNARD (12), and HARZ (17). From an examination of sections of the membranes used, and from a comparison made with the reports of these investigators, it is believed that the following structures are involved in these membranes: (1) an outer integument, a much compressed and hardly

distinguishable inner integument and nucellus, and a single layer of endosperm in *Arachis hypogaea* and *Prunus Amygdalus dulcis;* (2) a single integument and a layer of endosperm in *Xanthium pennsylvanicum* and *Juglans regia;* and (3) a portion of the integument, a layer of perisperm, and a layer of endosperm in *Cucurbita Pepo* and *C. maxima.* Details of the structure of these membranes, and a microchemical and chemical study of their composition will be given in a later paper.

Summary

1. Quantitative measurements were made of the permeability to water of certain non-living semipermeable plant membranes under experimentally controlled conditions.

2. The apparatus and method employed had the following advantages over osmometers ordinarily employed: (1) the passage of as small a quantity of water as 0.000337 gm. could be detected; (2) the exact area of the membrane used could be calculated; (3) the concentration of the solution exerting the osmotic pressure could be kept constant.

3. The effect of temperature upon the permeability to water of the seed coat of *Arachis hypogaea* was measured and the temperature coefficients for 10° rise in temperature were obtained. An average coefficient was not calculated. Since the temperature coefficients are not constant, but vary with the temperature, an average coefficient is without significance.

4. The temperature coefficient is lower than that according to the van't Hoff law, and is higher than the diffusion coefficient. There is no evidence that either chemical or physical processes are exclusively involved in the passage of water through the membrane.

5. The temperature coefficients showed higher values at lower temperatures and lower values at higher temperatures, and this is in agreement with the behavior of temperature coefficients in other processes.

6. A comparison is made with the temperature coefficients obtained in the permeability experiments of (1) KRABBE with living membranes, (2) RYSSELBERGHE with living membranes, (3)

BROWN and WORLEY with non-living seed coat membranes, (4) PFEFFER with copper ferrocyanide membrane.

7. No hysteresis or after effect of a previous temperature was observed.

8. It was found that the seed coats of peanut and almond showed a difference in permeability to water in opposite directions through the membrane, the faster rate being from the external toward the internal portion of the seed.

9. When distilled water was placed on one side of the membrane, the rate of water movement was proportional to the osmotic pressure applied upon the other side, when sodium chloride solutions were used; but this proportionality did not exist when cane sugar solutions were used.

10. When solutions of varying concentrations were placed on opposite sides of the membrane, it was found that the relation between rate and concentration difference was complex, and that in general equal osmotic differences do not necessarily produce equal rates; the rate is greatly affected by changes in the concentration of the internal solution; no mathematical relation was noted between the concentration on opposite sides and the rate of water movement through the membrane. The bearing of these facts upon plasmolytic experiments based on rate of water movement through membranes was pointed out.

11. A comparison of the permeability of several plant membranes under similar conditions was made, large differences appearing.

In conclusion, I wish to express my appreciation to Dr. WILLIAM CROCKER for suggesting the problem and rendering valuable assistance during the course of the experiments.

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